Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Hamdan FF, Gauthier J, Spiegelman D, et al. Mutations in *SYNGAP1* in autosomal nonsyndromic mental retardation. N Engl J Med 2009;360:599-605.

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Ethnic origin of the persons enrolled in the ASD, schizophrenia and control series - The parents of both the probands and the controls were asked to answer closed ended questions about the ethnic origin of each of their parents. The ASD series is composed of French Canadians (85), of other European Caucasians (54) and of non-Caucasians (3). The schizophrenia series is composed of European Caucasians (136) and Asians (7). The control series is composed of French Canadians (130), of other European Caucasians (37), of South Americans (4), of non-Caucasians (3) and of individuals of mixed origin (16).

Schizophrenia series - Schizophrenic subjects were selected from over 1000 schizophrenia families (2000 affected individuals) ascertained for genetic studies of schizophrenia, for which DNA samples were available. In order to ensure accurate diagnoses all individuals were evaluated by experienced investigators using the Diagnostic Interview for Genetic Studies (DIGS) or Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS), and multidimensional neurological, psychological, psychiatric, and pharmacological assessments at different centres. Family history for psychiatric disorders was also collected using the Family Interview for Genetic Studies (FIGS). All DIGS and FIGS have been reviewed by two or more psychiatrists for a final consensus diagnosis based on DSM-IIIR or DSM-IV at each centre. For all probands, specific inclusion criteria for the present study were: (1) The selected proband was definitely affected with SCZ only, not schizophreniform psychosis or bipolar depression with psychosis; (2) In families with multiple affected individuals, we selected the most severe SCZ case with early (<18 yrs) or childhood onset (<12 yrs), and/or additional neurodevelopmental problems, such as mental retardation, dyslexia and epilepsy, but not autistic disorder; for the COS series, mental retardation and epilepsy were exclusionary (3)

DNA from both parents was available, except for 8 proband samples, including 7 from Pakistan and one from Montreal, which were chosen from large multiplex families regardless of the availability of the parents' DNA; (4) Family history was well documented. Exclusion criteria included: (1) patients with psychotic symptoms mainly caused by alcohol, drug abuse, or other clinical diagnosis including major cytogenetic abnormalities; (2) patients with any of the four grandparents with Asian, African, Jewish, Arabic, Hispanic and American-Indian backgrounds were excluded. The final panel of the samples included 143 SCZ subjects from 5 different centres, i.e. 28 cases of childhood onset schizophrenia (COS) from NIMH, USA [1], 33 cases of adult onset SCZ from INSERM, France [2], 12 cases of adult onset SCZ from Montreal, Canada [3], 63 cases of adult onset familial SCZ from New York, USA [4], and 7 cases of adult onset SCZ selected from each of seven large highly consanguineous pedigrees with $\geq$ 10 affected individuals with schizophrenia and schizoaffective disorders from Pakistan. Detailed description of ascertainment strategy, diagnostic instrument and criteria was reported by each centre in previous publications, except for the Pakistani samples.

Autism series. Diagnostic and selection criteria for the ASD subjects are described in detail elsewhere [5]. Briefly all subjects were diagnosed using the Diagnostic and Statistical Manual of Mental Disorders criteria, and depending of the recruitment site, Autism Diagnostic Interview-Revised and the Autism Diagnostic Observation Schedule were used. In addition, the Autism Screening Questionnaire (ASQ) was also completed for all our subjects. We excluded patients with an estimated mental age < 18 months, a diagnosis of Rett Syndrome or Childhood Disintegrative Disorder and patients with evidence of any psychiatric and neurological conditions including: birth anoxia, rubella during pregnancy, fragile-X disorder, encephalitis, phenylketonuria, tuberous sclerosis, Tourette and West syndromes.

Clinical description of patients 1-3

Patient 1 is a girl, aged 4 years and 5 months. She was delivered at term after an unremarkable pregnancy. The APGAR score was 9^{1}, 9^{5} and 9^{10}. At birth, her weight was 4.0 kg (75^{th} percentile) and her head circumference was 34.5 cm (25^{th}-50^{th} percentile). Left congenital muscular torticollis with normal ultrasound

of the sternocleidomastoid was noted during the neonatal period. Motor development was characterized by some hypotonia. She started to walk at 2 years of age. Evaluation with the Bayley Scales of Infant Development III at 4 years and 2 months of age showed a developmental age of 22 months, consistent with moderate mental retardation (MR). Another series of assessments performed at 4 years and 5 months of age with the Mullen Scales of Early Learning (MSEL) and the Vineland Adaptive Behavioural Scale (VABS) confirmed the diagnosis of moderate MR (table 2). At that age, she knew less than 10 words and could not associate two words. Her vocal productions amounted to jabbers with inflection. She was able to respond to very simple instructions but often needed gesture to support the command. Shape discrimination and categorisation were still being acquired. She showed the ability to insert objects but not to retrieve them. Her digital dissociation was excellent but the use of a pencil was non efficient. Non-verbal social interactions were unremarkable.

Brief febrile and non-febrile tonic-clonic generalized seizures were first observed at 15 months of age. The patient had only one seizure over the last 2 years while being treated with topiramate. Ophthalmologic evaluation revealed the presence of strabismus. Height, weight and head circumference measurements were within normal values for girls of her age (table 2). On physical examination, no specific dysmorphic features were noticed, except for the presence of discrete left facial hypoplasia that is associated with a persistent left torticollis without limitations of cervical movements. Neurological examination revealed some hypotonia. Patient 1 was born to non-consanguineous South American parents. She has two older healthy maternal half-brothers. Family history is unremarkable.

Karyotyping (at a resolution of 500 bands), subtelomeric FISH studies and molecular testing for the triple repeat expansion associated with the Fragile X syndrome did not show any abnormality. Blood creatine kinase, lactate and ammonia levels were 125 U/L (reference values: 15-200 U/L), 1.00 mmol/L (reference values: 0,50 -2,20 mmol/L) and 31 μmol/L (reference values: 0-55 μmol/L), respectively. Plasma amino-acid chromatography was unremarkable. Electroencephalogram performed at 1 year and 11 months of age

revealed bi-occipital spikes during intermittent light stimulation. Brain MRI performed at the age of 2 did not reveal any abnormality.

Patient 2 is a girl, aged 5 years and 10 months. She was born at term after an unremarkable pregnancy. The APGAR score was 8^{1}, 9^{5} and 9^{10}. At birth, her weight was 3.6 kg (50^{th}-75^{th} percentile) and her head circumference was 35.7 cm (75^{th} percentile). The immediate neonatal course was uneventful. Motor development was characterized by some hypotonia. She started to walk at 21 months. She showed some drooling suggestive of some orofacial dyspraxia. Cognitive and behavioural assessments performed at 40 months of age with the Weschler Preschool and Primary Scale of Intelligence–III, the Griffiths Developmental Scale and the VABS were consistent with mild MR. Another series of assessments with the MSEL and the VABS performed at 70 months of age, when cognitive evaluation is more informative, showed moderate MR (table 2). At the time of the latter evaluation, her verbal production consisted in two word phrases. She could name approximately a dozen pictures and count to two. She was able to respond to simple commands but not when they comprised two unrelated actions. Identification was adequate for objects but basic object function was still being acquired. Form discrimination and object categorisation was present only for real objects. Her digital dissociation was adequate and she could easily insert pennies in a slot. She managed to stack 6 blocks and was able to imitate a four-block train. Her pencil holding was immature but functional. She drew a line but not a circle. Non-verbal social interactions were unremarkable.

Myoclonia and absences seizures were first noted at 2 years and 4 months of age. Bouts of seizures occurred twice a year while under treatment with valproic acid. Recurrence of seizures responded well to an increase of valproic acid dosage. Height, weight and head circumference measurements were within normal values for girls of her age (table 2). On physical examination, no dysmorphic features were noticed. Neurological examination revealed some hypotonia. Her parents are non-consanguineous French Canadians. She has a younger healthy sister. Family history is unremarkable.

Because of the hypotonia and the motor developmental delay, investigation of neuromuscular impairment was performed. Blood creatine kinase measurements (147 U/L; reference values: 50-200 U/L), electromyogram, study of nerve conduction velocities and molecular testing for the common expansion associated with Steinert myotonic dystrophy were normal. Karyotyping (at a resolution of 500 bands), subtelomeric FISH studies and molecular testing for the triple repeat expansion associated with the Fragile X syndrome did not show any abnormality. Electroencephalogram performed at 2 years and 6 months of age revealed bi-occipital spikes during intermittent light stimulation. Brain MRI performed at age 4 was normal.

Patient 3 is a girl, aged 12 years and 2 months. She was born at term after an unremarkable pregnancy. The APGAR score was 9^{1}, 9^{5} and 9^{10} and her birth weight was 2.8 kg (10^{th} percentile). The immediate neonatal course was uneventful. Motor development was characterized by some hypotonia. She started to walk at 2 years of age. Cognitive assessments performed at 8 years of age with the Columbia Mental maturity Scale and with the Stanford-Binet Intelligence Scales were consistent with moderate MR. Another series of assessments performed at 12 years and 2 months of age with the MSEL and the VABS confirmed the diagnosis of moderate MR (table 2) whereas evaluation with the Autism Diagnostic Observation Schedule was negative. At that age, her sentences were still incomplete. She named a dozen pictures and counted to 12. Her comprehension was adequate for simple questions and commands, but not when they comprised two unrelated actions. Her understanding of action verbs and object function was adequate. Color identification was possible but size, length and comparative concepts were not present. She was able to categorise by shape, type, color, and size. She showed an ability to spatially discriminate fine details but could only match 5 of 6 letters. Her digital dissociation was refined and she could spontaneously stacks more than 9 blocks, imitate a 4 blocks tower and sting beads. Her pencil holding was immature but functional. She drew a line and a circle upon imitation.

Height, weight and head circumference measurements were within normal values for girls of her age (table 2). On physical examination, no dysmorphic features were noticed. Neurological examination was

unremarkable. Her parents are non-consanguineous French Canadians. She has a younger healthy brother. Family history is negative for cognitive disorders.

Karyotyping (at a resolution of 550 bands), subtelomeric FISH studies and molecular testing for the triple repeat expansion associated with the Fragile X syndrome did not show any abnormality. Blood lactate and ammonia levels were 1.03 mmol/L (reference values: 0,50-2,20 mmol/L) and 5 µmol/L (reference values: 0-55 µmol/L), respectively. Plasma amino-acid chromatography was unremarkable. Brain CT-scan performed at 2 years of age was normal

Supplementary Table 1. Primer pairs used for PCR amplification of *SYNGAP1* exons and their intronic junctions.

Exon	Amplicon name	amplicon size (bp)	Forward Primer	Reverse Primer
1	G00223_054	355	GGTCTCGAGCCTCCATCCATC	TTTTCCCCAACCCAATCCTTCTAC
2	G00223_002	331	CTTGCCATTTTAGGCCTCTG	AGTCTCAATGGCCACCCTC
3	G00223_003	260	CTTCCTGGGAGGAGGCG	CAGCCCGGTCCATCTTC
4	G00223_004	245	GGGAACCTGGGTTAACAGC	TCTTTCTCAGACTCCTAGGGC
5	G00223_005	278	ATCCAGGGGCTCTCTACCAG	CCCCTCCCTCTGCATCTC
6	G00223_006	429	AAGTTGCAGCAAGCCGAG	CCTACCCTTTCCTCCAGTCC
7	G00223_007	252	GGGAGGAAGAGAAGGTAGCAG	ACTTTCCTCCCTAGGCCCC
8.1	G00223_055	348	ACCCAGTACACCGTCTCACC	GTCACGGTACAGATGCAGCC
8.2	G00223_060	242	TACTGTGAGCTCTGCCTGG	TGCTCTGTGAAGTGGCG
8.3	G00223_009	450	GAAGGACAAGGCAGGCTATG	GCCCTGTCCTCACTAACCC
9	G00223_010	296	AGTGAGGACAGGGCAAATTC	AAGCTGTGGAAGGGTGGAC
10	G00223_025	512	CAGATGTCCACCCCAGACC	AATTTGTCCCCATTCTGGTG
11	G00223_012	402	CTGGAAGCTGAGGGTCTCTG	AGACCCTTCTTGCCGACC
12	G00223_013	372	GGGAGGCTATGATACCTTGTG	AGGGTAGTTTCTCAGGCTCC
13	G00223_014	343	CTATCCCAACTCAGGCCCC	GGGCCCAGTGAGGAGTATC
14	G00223_015	200	CCGCCTCTCCTTTCATTTG	AGAGGAGTAGGGCGAAGGC

15.1	G00223_016	481	CCAGACCACAGCAAGGTTC	TCTGTGGTGACACCCATCTG
15.2	G00223_017	469	CGCTGACAGCAGCCTTG	AGCATGTGCTGCAGGTTG
15.3	G00223_032	698	CCCCCTGCTGCCTCCATCCTTCAT	AAGCCCCCAGCTGGCCCTATTCC
16	G00223_019	337	GTCTCCTTTGGCTGTGCTG	GGAAGTGACTAGAGATCTCCCC
17	G00223_020	379	ACAGGGATGGAGGCTGG	TTTGGGGATGGGAGTCAG
18	G00223_021	258	TCCAGAGAGCTATGGGGTTC	GCTAGGTGGCTGGTGTAGTG
19	G00223_022	316	CTATAGGGGAGGCCACTGC	ATGTCCAATCCTGGTGGTTG

Exons 8 and 15 were divided each into 3 overlapping amplicons.

Supplementary Table 2. Prediction of the functional effect of the missense mutations detected in *SYNGAP1* using the programs SIFT [6], PolyPhen [7], and SNAP [8].

Δ amino acid	SIFT score / prediction	PolyPhen score / prediction	SNAP % accuracy / prediction
D201E	1.00 / Tolerated	0.08 / Benign	92 / Neutral
T790N	0.49 / Tolerated	0.07 / Benign	69 / Neutral
R749Q	0.57 / Tolerated	1.36 / Benign	78 / Neutral
I1115T	0.59 / Tolerated	0.54 / Benign	60 / Neutral
P1283L	0.22 / Tolerated	0.00 / Benign	60 / Neutral
T1310M	1.00 / Tolerated	1.35 / Benign	85 / Neutral

Tolerated, benign, and neutral, indicate that the amino acid modification is unlikely to affect protein function.
SIFT: http://blocks.fhcrc.org/sift/SIFT.html
PolyPhen: http://genetics.bwh.harvard.edu/pph/
SNAP: http://cubic.bioc.columbia.edu/services/SNAP/

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